

Lund. Thesis, 1963.

(Sandberg, Nils

Experimental Studies

ON wound healing.)

THESIS

LUND

LANE

MEDICAL



LIBRARY

*California Medical Association
Fund*

Send. Thesis, 1963.
(With 6 preprints by the author.)

FROM THE INSTITUTE OF PHYSIOLOGY AND THE DEPARTMENT OF SURGERY, UNIVERSITY OF LUND,
LUND, SWEDEN

Thesis

EXPERIMENTAL STUDIES ON WOUND HEALING

BY

NILS SANDBERG

LANE MEDICAL LIBRARY
STANFORD UNIVERSITY
300 PASTEUR DRIVE
PALO ALTO, CALIF.

LUND 1963



Digitized by the Internet Archive
in 2026

https://archive.org/details/IA40153311_0174

FROM THE INSTITUTE OF PHYSIOLOGY AND THE DEPARTMENT OF SURGERY, UNIVERSITY OF LUND,
LUND, SWEDEN

EXPERIMENTAL STUDIES ON WOUND HEALING

BY

NILS SANDBERG

LANE MEDICAL LIBRARY
STANFORD UNIVERSITY
300 PASTEUR DRIVE
PALO ALTO, CALIF.

LUND 1963

CARL BLOMS BOKTRYCKERI A.-B.

The present publication is based on the following papers:

- I. The Tensile Strength of Healing Wounds and Collagen Formation in Rats and Rabbits. Together with B. Zederfeldt.
Acta Chirurgica Scandinavica, Vol. 126, 1963 (In press).
- II. On the Healing of Wounds in Rats during the Period of Cicatrization.
Acta Chirurgica Scandinavica, Vol. 126, 1963 (In press).
- III. Enhanced Rate of Healing in Rats with an Increased Rate of Histamine Formation.
Acta Chirurgica Scandinavica, Vol. 126, 1963 (In press).
- IV. Granulation Tissue Hydroxyproline in the Rat after Inhibition of Histamine Formation.
Acta Chirurgica Scandinavica, Vol. 126, 1963 (In press).
- V. Time Relationship between Administration of Cortisone and Wound Healing in Rats.
Acta Chirurgica Scandinavica, Vol. 126, 1963 (In press).
- VI. On the Effect of Cortisone on the Histamine Formation of Skin Wounds in the Rat. Together with C. Steinhardt.
Acta Chirurgica Scandinavica, Vol. 126, 1963 (In press).

In the following text the papers are referred to by the Roman figures I—VI.

INTRODUCTION

The repair of injured tissue, a fundamental and remarkable process with many problems in common with general tissue growth, has been studied from different aspects. However, the mechanisms remain obscure, above all those concerning the initiation and control of the processes involved. A wide variety of techniques have been used in studies of wound healing, ranging from visual inspection to methods using complicated instruments. Histological studies have contributed considerably to our knowledge of the healing process. The healing by "primary intention" of incised wounds has been shown to occur in essentially the same way as the healing by "secondary intention" of wounds with loss of substance. The difference in healing of these two types of wounds consists chiefly in the amount of newly formed tissue and in the duration of healing. (For details see AREY 1936, SANDBLOM 1944, LINDQUIST 1946, EDWARDS & DUNPHY 1958.)

Histological studies alone can hardly elucidate the functional capacity of the healing wound. The efficiency of healing can be studied by determinations of the tensile strength, *i.e.* by measuring the force necessary to rup-

ture the wound. PAGET (1853) measured the strength of healing divided Achilles tendons in rabbits. CLUMSKY (1899) investigated healing intestinal anastomoses with this method and described its principles. Techniques for determining the tensile strength of excised wounds in various tissues have since been developed by HOWES, SOOY & HARVEY (1929), SANDBLOM (1944) and CHARNEY, WILLIAMSON & BERNHART (1947). A method for recording the tensile strength of healing tissue *in situ* and *in vivo* was described by BOTSFORD (1941). SANDBLOM, PETERSEN & MUREN (1953) designed a tensiometer for this purpose and adapted the method for clinical use. Determination of the tensile strength with the wound *in situ* has the advantage that the wound is exposed to fewer manipulations before the measurement is made; especially in the early course of healing, manipulations may constitute a source of error.

The combination of measurements of the tensile strength of healing skin incisions with microscopical studies on wounds resulted in the recognition of three phases of healing: (1) A lag or preparatory phase lasting about 4 days in rats (HOWES, BRIGGS, SHEA & HAR-

VEY 1933) and 2 days in rabbits (SAND-BLOM 1944, ZEDERFELDT 1957). During this destructive and exudative phase the tensile strength is due mainly to the firmness of the clot. (2) A period of fibroplasia lasting until the 10th to 14th day in rats and rabbits. Granulation tissue with vascular endothelium and fibroblasts is seen. Formation of collagen fibrils and an increase of tensile strength occur (DUNPHY & UDUPA 1955). (3) A long period of cicatrization, during which the healing tissues differentiate with orientation and growth of collagen fibres (HOWES, HARVEY & HEWITT 1939).

Histochemical and chemical methods have contributed to our knowledge of the rate of collagen formation in healing incised wounds (for references see WILLIAMSON 1957). A clarification of collagen formation is important, since collagen is regarded to be mainly responsible for the tensile strength of healing wounds. Concurrent studies on the collagen formation and the tensile strength of wounds are scanty, but might be expected to bring valuable information. The amount of granulation tissue obtainable from incised wounds is generally too small to permit quantitative chemical analyses of tissue components, except by methods using radioactive isotopes (*e.g.* MOLTKE 1957, KAHLSON, NILSSON, ROSENGREN & ZEDERFELDT 1960, SAVLOV, STRAIN & HUEGIN 1962). Newly formed tissue can be obtained in larger amounts from superficial skin defects. Such tissue has been used for *e.g.* determination of its collagen content (ABER-CROMBIE, FLINT & JAMES 1954, DUNPHY

& UDUPA; GRILLO, WATTS & GROSS 1958). This means of producing newly formed granulation tissue for analyses has certain disadvantages. Thus, it is difficult to determine the margin of the newly formed tissue (EDWARDS, PERNOCKAS & DUNPHY 1957). In order to obtain newly formed tissue in adequate amounts other techniques have been applied. Subcutaneous injection of carrageenin (extract of *Chondrus crispus*) produces granulomatous tissue containing newly formed collagen (ROBERTSON 1952, ROBERTSON & SCHWARTZ 1953). After about 2 weeks, however, the granuloma begins to regress (JACKSON 1957).

Granulation tissue has also been shown to grow into implanted porous materials. MEIER, SCHULER & DESAULLES (1950) implanted cotton pellets subcutaneously and SCHILLING, FAVATA & RADAKOVICH (1953) inserted tantalum wire cylinders to obtain newly formed granulation tissue. Sponges made of plastic material were used in reconstructive surgery (GRINDLAY & WAUGH 1951). Granulation tissue grew into this material and it provided the means of sampling granulation tissue in sufficient amounts for analyses and also made longer observation periods possible. A method for obtaining newly formed tissue by subcutaneous implantation of polyvinyl sponges (IVALON^R) was described by BOUCEK & NOBLE (1955) and was modified by EDWARDS et al. (1957). The collagen content was determined chemically in tissue obtained in this way and it was found that collagen formation proceeded at the same rate as in granu-

lating skin defects (GOULD 1958). Histological examinations of sponge implants showed that granulation tissue, relatively poor in mobile cells, but rich in vascular endothelium and fibroblasts, grew into the polyvinyl sponges. A dense, collagenous tissue formed in the pores of the sponge and gradually filled the entire sponge. Not until about 4 months after implantation did signs of disintegration appear (LAPIÈRE 1961).

Chemical determination of the col-

lagen content in tissues has been made possible by the circumstance that the amino acid hydroxyproline appears to occur uniquely in collagen (reviews by RANDALL 1953, JACKSON 1958 and HARKNESS 1961). A widely used method of estimating hydroxyproline is that of NEUMAN & LOGAN (1950). Hydroxyproline is reported to be a constant part of collagen, constituting 13.6 per cent (BOWES, ELLIOTT & MOSS 1955).

PURPOSE OF THE PRESENT INVESTIGATION

Earlier investigations have shown that determinations of the tensile strength of healing skin incisions provide a suitable means for assessing the efficiency of healing. Changes in the rate of healing and in the tensile strength have been ascribed to altered collagen formation. Determination of hydroxyproline allows the measurements of changes in the collagen content of healing tissue. The nature of the relation between collagen formation and tensile strength of healing wounds is, however, unclear. The purpose of the present investigation was to study this relationship.

The main question studied was, to what extent changes in tensile strength are reflected by changes in collagen (hydroxyproline) content in granulation tissue in animals with a "normal" rate of healing and in animals with increased or decreased rate of healing.

The following topics were investigated:

- 1) The tensile strength of healing skin incisions and collagen (hydroxyproline) in granulation tissue of implanted polyvinyl sponges measured concurrently in the same animal during the first month of healing, when collagen formation is pronounced (I).
- 2) The tensile strength and collagen formation during the period of cicatrization. Attention was paid to questions concerning the relation between tensile strength of skin wounds and that of intact skin and to the relationship between the hydroxyproline concentration of intact skin and the tensile strength of skin and skin wounds (II).
- 3) The tensile strength and collagen formation under conditions of elevated or reduced rate of histamine formation with special reference to means of accelerating the rate of healing (III, IV).

- 4) The time relationship between administration of cortisone and rate of wound healing and collagen formation. An attempt was made

to clarify the question, whether cortisone exerts its effect on wound healing by an action on the rate of histamine formation (V, VI).

GENERAL PROCEDURES

Adult rats (about 6 months old) of either sex from a closed colony were used. Adult albino rabbits were also used in one type of experiments (I). The standard diets of the Institute were given, except when the rats received a partly synthetic diet (IV).

Operations on the rats were performed under anaesthesia with ether applied by open mask. The rabbits were anaesthetized with nembutal^R intravenously, supplemented by open mask ether.

Skin wounds were inflicted in a standardized way (I—III, V, VI). Detailed descriptions are given in papers I and III. After depilation of one side of the back the intended positions of wounds and sutures were marked. The skin of that side including the cutaneous muscle was incised with a sharp knife. Wounds were made parallel to the midline. In rats one 6 cm long incision, about 1.5 cm from the midline was made. In rabbits 3 incisions, each 4 cm long, were made 2.5 cm from one another and from the midline according to SANDBLOM (1944) and to ZEDERFELDT. Silk sutures were used.

In studies in which the healing of one wound on one side of the back was compared with that of another wound on the other side (III, V, VI),

the *control wound* was first inflicted and left to heal for the period studied. The *experimental wound* was inflicted on the other side after determinations had been made on the control wound.

The tensile strengths of control and experimental wounds were compared at the same intervals after wounding. The sutures were removed before testing. As a rule the tensile strength was determined *in vivo* with the wounds *in situ* (I, III, V) using the tensiometer described by SANDBLOM, PETERSEN & MUREN or a later modification of it. These tensiometers could not record tensile strength values exceeding 2000 g. When the tensile strength was expected to exceed this value, the healing wounds were excised (II, III) and their tensile strength was measured with a commercially manufactured tensiometer with a wider measuring range. This instrument was also used for measurements of the tensile strength of excised, cleansed non-wounded skin (II).

The tensile strength was determined on 2 or 3 segments of each wound and the mean value was taken as the tensile strength of the wound. The difference between the tensile strength of symmetrically situated segments of the experimental and the control wounds was expressed as a percentage

of the tensile strength of the corresponding segment of the control wound. The mean of the differences was given for each group of animals.

Determinations of hydroxyproline in newly formed granulation tissue were made in rats (I—V) and in rabbits (I). Tissue for analyses was obtained in polyvinyl sponges (IVALON^R) by a modification of BOUCEK & NOBLE's method. After depilation, a long incision was made near the midline and sponges, measuring $3 \times 1 \times 0.3$ cm, were placed longitudinally between the skin of the back and the underlying fascia at least 1 cm lateral to the incision which was finally sutured. In rats 2 or 3 and in rabbits 6 sponges were implanted on one side of the back. In the study described in paper I wounds were concurrently inflicted on the other. In paper II sponges were implanted on both sides simultaneously. In papers III—V a set of *control sponges* was first implanted on one side and removed after a predetermined period. After an interval of about 2 weeks the animals were treated and *experimental sponges* were implanted on the other side of the back via another incision and left for the same period as the control sponges.

A detailed description of the method used for determination of hydroxyproline is given in paper I. Sponges with newly formed granulation tissue were first cleansed mechanically and chem-

ically and then dried to constant weight. After hydrolysis and neutralization, hydroxyproline was determined colorimetrically according to NEUMAN & LOGAN. The values obtained were, as a rule, given both as hydroxyproline per unit of weight of newly formed dried tissue (concentration of hydroxyproline) and as hydroxyproline per unit of weight of dried implanted sponge (total amount of hydroxyproline). Determinations were also made of hydroxyproline in cleansed and dried pieces of intact skin (I, II).

The difference between the hydroxyproline values found of experimental and control sponges was expressed as a percentage of the value found of the control sponges. The mean of these percentages was given for each group of animals.

The significance of differences was assessed with Student's *t*-test. The differences were regarded as significant when $P < 0.05$.

In one study (VI) the rate of histamine formation in wound tissue of excised dorsal skin wounds and of non-wounded abdominal skin of rats was assessed *in vitro*. Minced tissue was incubated with ¹⁴C-histidine. The ¹⁴C-histamine formed was measured by the isotope dilution technique devised by SCHAYER (1956) as described by KAHLSON, ROSENGREN, WESTLING & WHITE (1958) and WHITE (1959).

Comments on General Procedures

Adult animals of approximately the same age were used for the investigation in order to minimize the effect of age. In the study on healing during the cicatrization period the age of the rats at the end of the experiments was at most one year (II). In this connection it should be mentioned that the concentration of hydroxyproline in non-wounded skin of older rats (II) was found to be higher than that of rats of about 6 months of age (I). CLAUSEN (1962) found the hydroxyproline concentration in human skin to increase with age.

Both male and female rats and rabbits were used (I—V), since the healing capacity was reported to be the same for both sexes (HARVEY & HOWES 1930). In the present investigation no differences regarding healing were observed between males and females. Female rats were preferred in one study (VI), as similar investigations of histamine formation were made on females (KAHLSON et al. 1960).

The experiments were carried out during all seasons of the year, but the animals were kept in a room at a temperature of 18—20°C. Changes in the ambient temperature have been demonstrated to influence the rate of healing both in cold-blooded (alligators) (EBELING 1922) and in warm-blooded animals (rabbits) (ERICI 1957, LUNDGREN, MUREN & ZEDERFELDT 1959). The skin was depilated immediately before each operation in order to get standardized conditions, since depilation was found to cause reduced

tensile strength of healing skin wounds, probably via reduced cutaneous circulation (SANDBLOM & MUREN 1954).

Control groups. In experiments on the effect of different factors on healing each animal served as its own control, because the rate of healing of skin wounds varies from one individual to another as has been shown by BOTSFORD, SANDBLOM (1944) and others.

The tensile strength of 7 day old healing skin incisions on one side of the back was not found to differ significantly from that of wounds inflicted on the other side at a later operation (III). The concentration and total amount of hydroxyproline in 7 day old granulation tissue of polyvinyl sponges implanted subcutaneously on one side of the back did not differ significantly from that obtained from sponges implanted on the other side at a later operation (III).

Effect of restricted food intake. With the exception of certain experiments with a restricted food intake the animals had free access to the standard diets (I—III, VI). On restriction of food intake to such an extent that the body weight was reduced by about 20 per cent of the initial body weight within 7 days, no significant change was found in the tensile strength of healing wounds (V). In cold-blooded animals (salamander) MORGAN (1906) found starvation to have no effect on wound healing. Restricted food intake for 4 days in rats with subsequent weight reduction of about 15 per cent

before the infliction of the wounds was reported not to affect the tensile strength (FIORE-DONATI & MOLTKE 1960). Complete withdrawal of food for 4 days before infliction of wounds followed by restricted food intake during a healing period of 7 days was, however, found to reduce the tensile strength (SANDBERG, unpublished). Withdrawal of food during a healing period of 7 days did not exert any effect on the rate of healing of stomach wounds in adult rats (HOWES et al. 1933). Neither did rationing of the

standard diet for 7 days in rats resulting in a 20 per cent weight reduction appear to alter the concentration of hydroxyproline in the granulation tissue of implanted polyvinyl sponges (IV). On the other hand, under this experimental condition the total amount of hydroxyproline increased, indicating that growth of tissue into subcutaneously implanted sponges was increased. Among contributing factors a reduction of the subcutaneous tissues altering the conditions for the ingrowth of tissue might be considered.

THE TENSILE STRENGTH OF HEALING WOUNDS AND COLLAGEN FORMATION

The investigation described in paper I consisted of a comparative study on rats and rabbits. The tensile strength of healing skin incisions and the formation of collagen (hydroxyproline) in newly formed granulation tissue of subcutaneously implanted polyvinyl sponges were determined at intervals varying from 5 to 30 days.

The tensile strength of skin wounds was found to be higher in rabbits than in rats during the entire experimental period which might be due to different thickness of the skin or to the shorter preparatory phase in rabbits. The tensile strength increased at about the same rate in both species during the first 2 weeks. In the rabbits it then continued to increase at the same rate as before and reached the upper limit of the tensiometer (2000 g) at about the 20th day. In rats the tensile

strength remained unchanged during the 3rd week. About the 30th day their tensile strength reached 2000 g.

The concentration of hydroxyproline in the granulation tissue of implanted polyvinyl sponges increased in both species most rapidly and at the same rate during the first 11 days. In rabbits it showed only slight changes from the 11th to the 15th day, but increased later and reached the hydroxyproline concentration of intact skin at about the 30th day. In rats it decreased between the 11th and 20th day and increased later; on the 30th day the concentration of hydroxyproline in the granulation tissue was about half of that in intact skin.

The total amount of hydroxyproline in granulation tissue increased in both species during the first 11 days. The values from rats were higher than

those from rabbits, indicating larger amount of tissue in sponges from rats. From the 11th to the 30th day the total amount of hydroxyproline in rabbits continued to increase, but at a somewhat slower rate than during the first 11 days. In rats no further increase occurred. Growth of tissue into the sponges, thus went on for a longer period in rabbits. The final values found for rabbits were higher than those for rats.

In rabbits a comparison of the rate of gain in wound tensile strength and in the concentration and total amount

of hydroxyproline in granulation tissue showed a correlation during the first 2 weeks. When the tensile strength exceeded the upper limit of the tensiometer, a comparison appeared less suitable. In rats the rate of gain in tensile strength and in concentration and total amount of hydroxyproline seemed to be correlated up to the 20th day. Determinations of wound tensile strength and of hydroxyproline in granulation tissue seemed to be suitable means in studies of the rapid phase of collagen formation.

ON THE HEALING OF WOUNDS DURING THE PERIOD OF CICATRIZATION

Our knowledge of the tensile strength of skin wounds during the period of cicatrization is incomplete. The investigation described in paper II comprised a study of the tensile strength of excised skin wounds after healing periods of 1 to 5 months. The tensile strength of excised skin wounds was found to increase continuously during the first 4 months. During the 5th month no further increase occurred. The tensile strengths of healing wounds recorded during the experimental period did not reach the strength of excised non-wounded skin and did not seem to be correlated to it.

The hydroxyproline concentration of non-wounded skin was determined. The tensile strength of excised non-wounded skin and that of excised skin wounds did not seem to vary with the hydroxyproline concentration of adja-

cent non-wounded skin. This suggested that the amount of collagen (hydroxyproline) might be of less importance than the quality of the collagen for the tensile strength of skin and cicatrizing wounds.

The concentration of hydroxyproline in granulation tissue of sponges implanted for 1.5 to 4 months was found to be highest after the shortest implantation period. After 2 months it decreased and remained fairly constant for the rest of the period studied. A correlation between the tensile strength of skin wounds and the hydroxyproline concentration in sponge implants was not obvious during the studied part of the cicatrization period. The formation of collagen in the sponges seemed to be completed or a balance between formation and decomposition to be attained.

ENHANCED RATE OF WOUND HEALING

Acceleration of wound healing has been widely studied (for ref. see SANDBLOM 1944, 1949, EDWARDS & DUNPHY). Several agents have been recommended for this purpose. Mostly, they seemed only to have a normalizing effect on a reduced rate of healing. When an acceleration of the rate of healing was observed, the effect could often be ascribed to non-specific reactions. CARREL (1921) showed mild infection to favour the healing of open wounds, while application of a "non-irritant dressing" prevented healing. BOTSFORD found mild infection to increase the tensile strength of healing skin wounds. Comparative studies on germ-free and "conventionalized" guinea-pigs appear to have confirmed BOTSFORD's findings (LEVENSON & TENNANT 1963).

Bacterial endotoxin administered locally or systemically in suitable doses was found to stimulate healing in guinea-pigs. The collagen content of subcutaneously implanted sponges was increased, but no measurable change was noted in the tensile strength of excised skin wounds (FREIHOFFER, ROBINSON, JACKSON & DUNPHY 1960).

Local administration of powdered cartilage in a closed wound was found to increase the tensile strength (PRUDEN, NISHIHARA & BAKER 1957) and was reported to enhance healing of ulcers produced by intradermal injection of croton-oil (HOUCK, JACOB, DE ANGELO & VICKERS 1962). The effect of this substance could possibly be due

to a foreign body reaction, but its mode of action is still unknown.

A new approach to the possibility of accelerating the rate of healing was revealed during studies on histamine. An association was postulated between the rate of histamine formation and certain types of rapid tissue growth (see KAHLSON 1962 a, b). In wound tissue from healing skin incisions in rats the capacity to form histamine was found to be markedly increased as against non-wounded skin (KAHLSON et al. 1960). An elevated rate of histamine formation can be experimentally induced. Repeated injections of the histamine releasers (see PATON 1957), compound 48/80¹ and polymyxine B² were shown to elevate the rate of histamine formation in the skin of the rat (SCHAYER, ROTSCCHILD & BIZONY 1959, SCHAYER & GANLEY 1959, KAHLSON et al. 1960). KAHLSON et al. (1960) found increased tensile strength in 5 day old skin incisions after preoperative treatment with these drugs. Repeated subcutaneous injections of long-acting histamine (histamine-dipicrate), on the other hand, did not appear to affect the tensile strength of 5 day old wounds. These findings suggested that the intracellular histamine formation is of greater importance than the resulting extracellular histamine (KAHLSON 1962 b).

In the study described in paper III an attempt was made to accelerate the

¹ A condensation product of p-methoxyphenetyl-methylamine and formaldehyde.

² Polymyxin B sulfat (Pfizer).

rate of wound healing and collagen formation. Results of part of this investigation have been published earlier (SANDBERG 1962). The rats were given compound 48/80 (1 mg per kg per day) intraperitoneally for 3 days beginning after determination of the tensile strength of the control wounds. Experimental wounds were thus made in the skin of rats, whose histamine forming capacity was increased. Rats treated in this way showed an increased wound tensile strength. Statistical analysis of the mean percentage differences of the tensile strength of experimental and control wounds revealed a significant increase of the tensile strength of experimental wounds after healing periods of 3, 5, 7, 10, 12 and 15 days. However, after 25 and 30 days' healing no differences in tensile strength were found.

Thus, an increased tensile strength was obtained in these rats as early as in the preparatory phase assumed to be 4 days (HOWES et al. 1933). The increase was still apparent during the fibroplasia phase, known to be the phase of collagen formation in healing wounds (DUNPHY & UDUPA). During the studied part of the cicatrization period the tensile strength returned to normal. The findings appeared to imply that an accelerated rate of healing of skin incisions was obtained after preoperative injections of compound 48/80.

After administration of compound 48/80 tissue of polyvinyl sponges implanted subcutaneously in rats for 3, 5, 7 or 9 days contained an increased

concentration and total amount of hydroxyproline compared with tissue of control sponges. The mean percentage differences of the concentration of hydroxyproline between granulation tissue of experimental and control sponges were significantly larger after 5, 7 and 9 days' implantation. The total amount of hydroxyproline was significantly increased after periods of 3, 5, 7 and 9 days. After 11 and 13 days, however, the hydroxyproline in the granulation tissue in experimental sponges did not differ from control sponges in the same animal.

In some experiments polymyxine B, a histamine liberator different chemically from 48/80 (PATON), was given intraperitoneally (2 mg per kg per day) for 3 days before the implantation of experimental sponges. On the 7th and the 9th day both the concentration and the total amount of hydroxyproline in granulation tissue were found to be significantly increased.

The increase in concentration and total amount of hydroxyproline recorded in granulation tissue after inducing an increase in the rate of histamine formation, thus occurred during a period covering the preparatory phase and the early part of the fibroplasia period of healing skin wounds. These findings indicated that the relationship found in untreated animals (DUNPHY & UDUPA; I) between tensile strength of skin wounds and of hydroxyproline in granulation tissue, also held, when the rate of wound healing was accelerated.

GRANULATION TISSUE HYDROXYPROLINE AFTER INHIBITION OF HISTAMINE FORMATION

Inhibition of histamine formation was shown to occur in rats fed a pyridoxine deficient diet and given injections of semicarbazide¹ (KAHLSON & ROSENGREN 1959). In rats treated in this way, the tensile strength of 5 day old healing wounds was found to be reduced. A pyridoxine deficient diet alone did not appear to change the tensile strength (KAHLSON et al. 1960). Neither did subcutaneous injections of semicarbazide alone influence rapidly growing tissue which was studied in the foetus of the rat (KAHLSON & ROSENGREN).

Paper IV deals with the effect of injections of semicarbazide combined with a pyridoxine deficient diet on the formation of hydroxyproline in newly formed granulation tissue in polyvinyl sponge implants in rats. A partly synthetic diet with an adequate vitamin content (according to GUSTAFSSON 1959), was given when the control sponges lay implanted. After removal of these sponges this diet was continued until one week before implantation of the experimental sponges. At this time pyridoxine was omitted from the diet. During the following week, when the rats were carrying their experimental sponges, subcutaneous injections of semicarbazide (50 mg per kg body weight twice daily) were given. The treatment suppressed histamine formation in rats by about 80 per cent, as judged from the urinary excretion of histamine (KAHLSON, ROSENGREN &

THUNBERG 1963). This treatment together with the operative procedures used, resulted in a loss of body-weight of 15 per cent within 7 days. Therefore, the body weight was maintained by tube feeding in rats subjected to an inhibition of histamine formation. In these rats the concentration and total amount of hydroxyproline in the granulation tissue of sponges implanted for 7 days were found to be significantly reduced. After termination of enzyme inhibition a rebound phase with increased histamine formation was found (KAHLSON et al. 1960). During the rebound phase the concentration and total amount of hydroxyproline were found to be significantly elevated in the present study.

Thus, reduced concentration and total amount of hydroxyproline in granulation tissue of implanted polyvinyl sponges were found in rats with inhibited histamine formation. When the rate of histamine formation was elevated the concentration and total amount of hydroxyproline were increased (III, IV). The wound tensile strength was found to be reduced, respectively, increased under these experimental conditions (KAHLSON et al. 1960; III). This appeared to suggest a relationship between collagen (hydroxyproline) formation and wound tensile strength in conditions with induced changes in the rate of histamine formation. The findings supported the view that histamine formation and certain types of tissue growth are associated (KAHLSON).

¹ Carbamylhydrazine HCl.

SOME ASPECTS OF CORTISONE AND WOUND HEALING

The mechanism of the inhibiting effect of large doses of cortisone on wound healing has not been clarified. Papers V and VI are concerned with an investigation of the effects of cortisone on wound healing and on the rate of histamine formation in skin and skin wounds. In order to study the problem it appeared advisable first to ascertain at what stage of healing cortisone exerted its effect.

Cortisone¹ in a water suspension was given intramuscularly to rats in a daily single dose of 10 mg (about 4 mg per 100 g body weight). The inhibitory effect of cortisone on the healing of 7 day old skin wounds was found to vary with the interval between the day of infliction of the wounds and the day the injections were started. The tensile strength of healing wounds was significantly reduced in experiments in which cortisone was given during the healing period with the first injection the same day as, or at most one day after infliction of the wounds. When the wounds were allowed to heal for the first 2 days or longer without administration of cortisone to the rats, the tensile strength was not significantly reduced in 7 day old skin wounds. Similar results have been obtained in studies on the healing of superficial skin defects in mice (SPAIN, MOLOMUT & HABER 1950).

Cortisone treated rats showed a loss of body weight. The loss was largest for rats treated during the entire period

of healing of the experimental wounds (13 per cent in 7 days). Restriction of food intake resulting in a weight loss of the same order was not followed by reduced tensile strength of healing wounds (V).

Treatment for 3 days with cortisone before infliction of experimental wounds resulted in a significantly reduced tensile strength of 5 and 7 day old wounds. On the other hand, pretreatment with cortisone followed by 3 days without cortisone before infliction of the experimental wounds, appeared to have no effect on the tensile strength.

It thus appeared that cortisone exerted its effects during the early course of healing.

Hydroxyproline in granulation tissue of polyvinyl sponge implants was also studied. In rats pretreated for 3 days with cortisone it was found that the concentration and total amount of hydroxyproline were significantly reduced in granulation tissue of sponges after 5 and 7 days' implantation. The same type of cortisone treatment which was followed by a reduction of the tensile strength of healing wounds, was thus found to decrease the concentration and total amount of hydroxyproline in granulation tissue. Consequently, the tensile strength and hydroxyproline formation seemed to be connected also in cortisone-treated rats.

Previous investigations on rats have shown that injections of cortisone inhibited histamine formation in intact skin *in vivo* and *in vitro* (SCHAYER,

¹ 11-dehydro-17-hydroxycorticosteroneacetate.

SMILEY & DAVIS 1954, SCHAYER, DAVIS & SMILEY 1955). The observations that (1) tissues of an excised skin wound showed a more rapid rate of formation of histamine than non-wounded skin (KAHLSON et al. 1960) and, (2) the rate of wound healing could be increased or decreased, respectively, at experimentally elevated or lowered rate of histamine formation (KAHLSON et al. 1960; III, IV), seemed to indicate a relationship between the reduced rate of healing after cortisone treatment (V) and the rate of histamine formation.

In the investigations described in paper VI the rate of histamine formation in rats was determined using an isotope dilution technique, in 24 and 48 hour old wound tissue consisting of excised dorsal skin wounds. The rate of histamine formation was found to be markedly increased in wound tissue as compared with that of non-

wounded abdominal skin (8 rats), in confirmation of the findings by KAHLSON et al. (1960). Treatment with cortisone for 3 days before infliction of the wounds resulted in a decrease of the rate of histamine formation in tissues from 24 and 48 hour old wounds, compared with control wounds from the same animal. However, in comparison to that of non-wounded skin, the rate of histamine formation in wound tissue was increased also in cortisone treated animals. The findings by SCHAYER et al. (1954, 1955) that the rate of formation of histamine is decreased in intact skin after administration of cortisone was supported.

These studies on the effects of cortisone together with the findings described in papers III and IV appeared to suggest that cortisone exerted its effects on wound healing by an action on histamine formation.

GENERAL SUMMARY AND CONCLUSIONS

The relationship between the tensile strength of healing skin wounds and the collagen (hydroxyproline) formation in granulation tissue grown into polyvinyl sponges was investigated. The study was performed on untreated rabbits and rats and on rats treated in such a way that a decrease or an increase of the rate of wound healing occurred.

From the 5th to the 13th day the wound tensile strength and the collagen (hydroxyproline) content in granulation tissue increased at the same rate. This

occurred in both rats and rabbits and indicated a correlation between the tensile strength and the collagen formation. During the 3rd and 4th week after wounding such a relationship was not found; the tensile strength showed a further increase, while the hydroxyproline content showed only slight changes. It was concluded that determinations of wound tensile strength and hydroxyproline in sponge implants were suitable means to study the process of wound healing during the phase of rapid collagen formation.

Wound tensile strength was higher in rabbits than in rats, but the rate of gain in tensile strength was roughly the same from the 5th to the 13th day. A further increase was noted in rabbits during the 3rd week and in rats during the 4th week.

During the period of cicatrization studied in rats, the tensile strength of skin wounds showed an increase up to the 4th month. During the 5th month no gain in the tensile strength occurred. At the end of this period the tensile strength of wounds was still less than that of intact skin. There was neither a correlation between the tensile strength of wounds and that of intact skin, nor between the concentration of hydroxyproline of intact skin and the tensile strength of wounds or intact skin.

The tensile strength of healing wounds and the hydroxyproline content in sponge implants were increased when the rats were treated preoperatively with histamine releasers known to induce an increased rate of histamine formation. Wound healing was accelerated during the fibroplasia period returning later to a normal rate. The acceleration might be due to a promotion of early collagen formation. The observations appeared to support the notion (KAHLSON 1960) that histamine forming capacity and rapid tissue growth are related.

When the rats received a pyridoxine-deficient diet and injections of semicarbazide, a procedure known to inhibit the rate of histamine formation, the hydroxyproline content in granulation tissue was decreased. During the

rebound phase of histamine formation which occurs after termination of the inhibition, the hydroxyproline content was increased. KAHLSON et al. (1960) observed corresponding changes in the tensile strength of healing wounds. Thus, a correlation appeared to exist between the tensile strength and hydroxyproline formation during and after inhibition of histamine formation.

A study of the time relationship between administration of cortisone and wound healing showed that the tensile strength was reduced, when cortisone was given during the entire healing period (7 days). The tensile strength was not reduced, when cortisone treatment was started 2 days or later after wound infliction. Treatment for 3 days before infliction of wounds, reduced wound tensile strength and hydroxyproline content in sponge implants. Pretreatment followed by 3 days without cortisone before wound infliction did not influence the tensile strength. The findings indicated that the inhibitory effect of cortisone on healing was exerted during the early stage of healing.

Wound tissue showed an elevated rate of histamine formation which supported earlier findings. Pretreatment with cortisone reduced the elevated rate of histamine formation of 24 and 48 hour old wounds. It was suggested that cortisone might exert its effect on wound healing by an action on histamine formation.

The investigation was supported by grants from the Medical Faculty, University of Lund and Maggie Stephens' Foundation.

REFERENCES

- ABERCROMBIE, M., FLINT, M. H. & JAMES, D. W.: Collagen formation and wound contraction during repair of small excised wounds in the skin of rats. *J Embryol Exp Morph* 2: 264—274, 1954.
- AREY, L. B.: Wound healing. *Physiol Rev* 16: 327—406, 1936.
- BOTSFORD, T. W.: The tensile strength of sutured skin wounds during healing. *Surg Gynec Obstet* 72: 690—697, 1941.
- BOUCEK, R. J. & NOBLE, N. L.: Connective tissue. A technique of its isolation and study. *AMA Arch Path* 59: 553—558, 1955.
- BOWES, J. H., ELLIOTT, R. G. & MOSS, J. A.: The composition of collagen and acid-soluble collagen of bovine skin. *Biochem J* 61: 143—150, 1955.
- CARREL, A.: Cicatrization of wounds. XII. Factors initiating regeneration. *J Exp Med* 34: 425—434, 1921.
- CHARNEY, J., WILLIAMSON, M. B. & BERNHART, F. W.: An apparatus for the determination of the tensile strength of healing wounds. *Science* 105: 396—397, 1947.
- CLAUSEN, B.: Influence of age on connective tissue. Hexosamine and hydroxyproline in human aorta, myocardium and skin. *Lab Invest* 11: 229—234, 1962.
- CLUMSKY, V.: Experimentelle Untersuchungen über die verschiedenen Methoden der Darmvereinigung. *Brun Beitr Klin Chir* 25: 539—600, 1899.
- DUNPHY, J. E. & UDUPA, K. N.: Chemical and histochemical sequences in the normal healing of wounds. *New Engl J Med* 253: 847—851, 1955.
- EBELING, A. H.: Cicatrization of wounds. XIII. The temperature coefficient. *J Exp Med* 35: 657—659, 1922.
- EDWARDS, L. C. & DUNPHY, J. E.: Wound healing. I. Injury and normal repair. *New Engl J Med* 259: 224—233, 1958.
- EDWARDS, L. C. & DUNPHY, J. E.: Wound healing. II. Injury and abnormal repair. *New Engl J Med* 259: 275—285, 1958.
- EDWARDS, L. C., PERNOKAS, L. N. & DUNPHY, J. E.: The use of a plastic sponge to sample regenerating tissue in healing wounds. *Surg Gynec Obstet* 105: 303—309, 1957.
- ERICI, I.: Effect of environmental temperature on the rate of wound healing. *Acta Chir Scand* 112: 345—347, 1957.
- FIGLIORE-DONATI, L. & MOLTKE, E.: Effect of reserpine, 5-hydroxytryptamine and polymyxin B on mast cells and tensile strength of healing wounds. *Acta Endocr* 34: 430—436, 1960.
- FRIEDHOFFER, U., ROBINSON, E. A., JACKSON, D. S. & DUNPHY, J. E.: The effect of bacterial endotoxin on connective tissue growth and wound tensile strength. *Surg Forum* 11: 293—295, 1960.
- GOULD, B. S.: Biosynthesis of collagen. III. The direct action of ascorbic acid on hydroxyproline and collagen formation in subcutaneous polyvinyl sponge implants in guinea pigs. *J Biol Chem* 232: 637—649, 1958.
- GRILLO, H. C., WATTS, G. T. & GROSS, J.: Studies in wound healing: I. Contraction and the wound contents. *Ann Surg* 148: 145—152, 1958.
- GRINDLAY, J. H. & WAUGH, J. M.: Plastic sponge which acts as a framework for living tissue; experimental studies and preliminary report of use to reinforce abdominal aneurisms. *AMA Arch Surg* 63: 288—297, 1951.
- GUSTAFSSON, B.: Lightweight stainless steel systems for rearing germfree animals. *Ann N Y Acad Sci* 78: 17—28, 1959.
- HARKNESS, R. D.: Biological functions of collagen. *Biol Rev* 36: 399—463, 1961.
- HARVEY, S. C. & HOWES, E. L.: Effect of high protein diet on the velocity of growth of fibroblasts in the healing wound. *Ann Surg* 91: 641—650, 1930.
- HOUCK, J. C., JACOB, R. A., DE ANGELO, L. & VICKERS, K.: The inhibition of inflammation and the acceleration of tissue repair by cartilage powder. *Surgery* 51: 632—638, 1962.
- HOWES, E. L., BRIGGS, H., SHEA, R. & HARVEY, S. C.: Effect of complete and partial starvation on the rate of fibroplasia in the

- healing wound. *AMA Arch Surg* 27: 846—858, 1933.
- HOWES, E. L., HARVEY, S. C. & HEWITT, C.: Rate of fibroplasia and differentiation in the healing of cutaneous wounds in different species of animals. *AMA Arch Surg* 38: 934—945, 1939.
- HOWES, E. L., SOOY, J. W. & HARVEY, S. C.: The healing of wounds as determined by their tensile strength. *JAMA* 92: 42—45, 1929.
- JACKSON, D. S.: Connective tissue growth stimulated by carrageenin. I. The formation and removal of collagen. *Biochem J* 65: 277—284, 1957.
- JACKSON, D. S.: Some biochemical aspects of fibrogenesis and wound healing. *New Engl J Med* 259: 814—820, 1958.
- KAHLSON, G.: A place for histamine in normal physiology. *Lancet* 1: 67—71, 1960.
- KAHLSON, G.: New approaches to the physiology of histamine. *Perspect Biol Med* 5: 179—197, 1962 a.
- KAHLSON, G.: Nascent Histamine and Methods of its Estimation. *Proc Internat Un Physiol Sci. XXII Internat Congr Leiden* 1: 856—862, 1962 b.
- KAHLSON, G., NILSSON, K., ROSENGREN, E. & ZEDERFELDT, B.: Wound healing as dependent on rate of histamine formation. *Lancet* 2: 230—234, 1960.
- KAHLSON, G. & ROSENGREN, E.: Prevention of foetal development by enzyme inhibition. *Nature* 184: 1238—1239, 1959.
- KAHLSON, G., ROSENGREN, E. & THUNBERG, R.: Observations on inhibition of histamine formation. *J Physiol (Lond)* (In press).
- KAHLSON, G., ROSENGREN, E., WESTLING, H. & WHITE, T.: The site of increased formation of histamine in the pregnant rat. *J Physiol (Lond)* 144: 337—348, 1958.
- LAPIÈRE, C. M.: Etude histologique du développement du tissu conjonctif au niveau d'éponges en Ivalon implantées chez le rat. *Arch Belg Derm Syphiligr*, 17: 135—147, 1961.
- LEVENSON, M. & TENNANT, B.: Some metabolic and nutritional studies with germ-free animals. *Fed Proc* 22: 109—119, 1963.
- LINDQUIST, G.: The Healing of Skin Defects. An Experimental Study on the White Rat. *Acta Chir Scand Suppl* 107, 1946.
- LUNDGREN, C., MUREN, A. & ZEDERFELDT, B.: Effect of coldvasoconstriction on wound healing in the rabbit. *Acta Chir Scand* 118: 1—4, 1959.
- MEIER, R., SCHULER, W. & DESAULLES, P.: Zur Frage des Mechanismus der Hemmung des Bindegewebswachstums durch Cortisone. *Experientia* 6: 469—471, 1950.
- MOLTKE, E.: Uptake of ^{35}S -sulphate by healing wounds. Effect of thyroxine and of ascorbic acid deficiency. *Acta Endocr* 25: 179—186, 1957.
- MORGAN, T. H.: The physiology of regeneration. *J Exp Zool* 3: 457—500, 1906.
- NEUMAN, R. E. & LOGAN, M. A.: The determination of hydroxyproline. *J Biol Chem* 184: 299—306, 1950.
- PAGET, J.: Lectures on Surgical Pathology. London 1853, in CAMERON, G. R.: *The Pathology of the Cell*. Edinburgh, Oliver & Boyd 1952.
- PATON, W. D. M.: Histamine release by compounds of simple chemical structure. *Pharm Rev* 9: 269—328, 1957.
- PRUDDEN, J. F., NISHIHARA, G., & BAKER, L.: The acceleration of wound healing with cartilage-I. *Surg Gynec Obstet* 105: 283—286, 1957.
- RANDALL, J. T.: *Nature and Structure of Collagen*. London, Butterworths Scientific Publications 1953.
- ROBERTSON, W. VAN B.: The effect of ascorbic acid deficiency on the collagen concentration of newly induced fibrous tissue. *J Biol Chem* 196: 403—408, 1952.
- ROBERTSON, W. VAN B. & SCHWARTZ, B.: Ascorbic acid and the formation of collagen. *J Biol Chem* 201: 689—696, 1953.
- SANDBERG, N.: Accelerated collagen formation and histamine. *Nature* 194: 183, 1962.
- SANDBLOM, PH.: The Tensile Strength of Healing Wounds. An Experimental Study. *Acta Chir Scand Suppl* 89, 1944.
- SANDBLOM, PH.: The effect of injury on wound healing. *Ann Surg* 129: 305—314, 1949.
- SANDBLOM, PH. & MUREN, A.: Differences between the rate of healing of wounds in-

- flicted with short time interval. *Ann Surg* 140: 449—458, 1954.
- SANDBLOM, PH., PETERSEN, P. & MUREN, A.: Determination of the tensile strength of the healing wounds as a clinical test. *Acta Chir Scand* 105: 252—257, 1953.
- SAVLOV, E. D., STRAIN, W. H. & HUEGIN, F.: Radiozinc studies in experimental wound healing. *J Surg Res* 2: 209—212, 1962.
- SCHAYER, R. W.: Formation and binding of histamine by rat tissues *in vitro*. *Amer J Physiol* 187: 63—65, 1956.
- SCHAYER, R. W., DAVIS, K. J. & SMILEY, R. L.: Binding of histamine *in vitro* and its inhibition by cortisone. *Amer J Physiol* 182: 54—56, 1955.
- SCHAYER, R. W. & GANLEY, O. H.: Adaptive increase in mammalian histidine decarboxylase activity in response to nonspecific stress. *Amer J Physiol* 197: 721—724, 1959.
- SCHAYER, R. W., ROTSCILD, Z. & BIZONY, P.: Increase in histidine decarboxylase activity of rat skin following treatment with compound 48/80. *Amer J Physiol* 196: 295—298, 1959.
- SCHAYER, R. W., SMILEY, R. L. & DAVIS, K. J.: Inhibition by cortisone of the binding of new histamine in rat tissues. *Proc Soc Exp Biol Med* 87: 590—592, 1954.
- SCHILLING, J. A., FAVATA, B. V. & RADAKOVICH, M.: Studies of fibroplasia in wound healing. *Surg Gynec Obstet* 96: 143—149, 1953.
- SPAIN, D. M., MOLOMUT, N. & HABER, A.: The effect of cortisone on the formation of granulation tissue in mice. *Amer J Pathol* 26: 710—715, 1950.
- WHITE, T.: Formation and catabolism of histamine in brain tissue *in vitro*. *J Physiol (Lond)* 149: 34—42, 1959.
- WILLIAMSON, M. B.: *The Healing of Wounds. A Symposium on Recent Trends and Studies*. New York, The Blakiston Division, Mc Graw-Hill Book Company, Inc. 1957.
- ZEDERFELDT, B.: *Studies on Wound Healing and Trauma with Special Reference to Intravascular Aggregation of Erythrocytes*. *Acta Chir Scand Suppl* 224, 1957.

EXPERIMENTAL STUDIES ON WOUND HEALING

AKADEMISK AVHANDLING

SOM MED VEDERBÖRLIGT TILLSTÅND AV MEDICINSKA FAKULTETEN I LUND
FÖR VINNANDE AV MEDICINE DOKTORSGRAD KOMMER ATT OFFENTLIGEN
FÖRSVARAS Å FYSIOLOGISKA INSTITUTIONENS FÖRELÄSNINGSSAL
ONSDAGEN DEN 15 MAJ 1963 KL. 9.15 (PRECIS)

AV

NILS SANDBERG
MED. LIC., VG.

LUND 1963
CARL BLOMS BOKTRYCKERI A.-B.

29 1966

L 2 6 1967

THESIS

LANE MEDICAL LIBRARY
STANFORD UNIVERSITY
300 PASTEUR DRIVE
PALO ALTO, CALIF.

LANE MEDICAL LIBRARY
STANFORD UNIVERSITY

This book should be returned on or before
the date last stamped below.

JUL 16 1963

NOV 21 1963

MAR 21 1964

MAY 8 - 1964

DEC - 4 1964

FEB 18 1967

25M-3-58-88267

THESIS

Lund Thesis, 1963

(Sandberg, Nils.

Experimental studies

NAME wound healing DATE DUE

DEMCO

